The Indiana Department of Environmental Management through the NPDES permitting program specifies that municipal NPDES permits for treatment plants that are over 1 MGD, discharge into a lake or sink hole or utilize UV disinfection are required to report and monitor *E. coli* levels during those seasons of the year when the potential exists for partial and full body contact with those waters.

In as much as 40 CFR 136 does not list methods for *E. coli* analysis, IDEM had designated the EPA recommended method: the mTEC agar, membrane filtration method as the procedure that NPDES permittees should use. The mTEC method depends on lactose fermentation metabolism and utilizes colorimetric indicators of pH changes. The procedure is time-consuming to prepare. The agar can be purchase in dehydrated form and must be made up at the laboratory, it has a short shelf life and must be made in small quantities. The substrate, too, must be made in the lab. This time consuming process is not a problem for large plants that have adequate personnel for any laboratory procedure, but for smaller plants that may have one lab person or a part-time lab person, the m-TEC procedure occupies a large amount of time. Analyzing the results can be difficult, even for experienced technicians, in differentiating the *E. coli* from general coliform and other background groups with confidence.

Micrology Laboratories in Goshen, Indiana has developed a membrane filtration recovery technique for *E. coli* that is easier, quicker and less subjective to interpret than the m-TEC procedure. The COLISCAN MFh medium utilizes chromogenic substrates that measure enzymatic activity. The COLISCAN MFh media can be purchased commercially and stored frozen for up to six months. Laboratory personnel need only keep a usable amount thawed and pipette the media onto a pad in the petri dish. No substrate is necessary, consequently, there is no substrate preparation step. No resuscitation step is necessary, thus eliminating the two hour changeover step that the m-TEC method requires. With the COLISCAN MFh the *E. coli* appear as blue, non-*E. coli* coliforms appear as pink and non-coliform colonies appear as white or translucent, these easily discernable differences allow the technician to count the plate with a greater degree of confidence.

The following breakdown gives a detailed comparison of the two above mentioned membrane filtration methods and outlines the steps required for the preparation of both the mTEC and the COLISCAN MFh methods:

mTEC	COLISCAN MFh
<u>RECOVERY METHOD</u>	<u>RECOVERY METHOD</u>
lactose fermentation metabolism (utilizes colorimetric indicators of pH changes resulting from these acidic productions)	Measures enzymatic activity (utilizes two chromogenic substrates)

MEDIA

Use dehydrated mTEC agar. Store in desiccator if possible. Has short shelf life. Make up only enough to last two weeks. Dissolve media in lab grade water in a beaker, cover with foil and bring to boil. Immediately put media into autoclave and set timer for 15 minutes, slow exhaust. Total cycle should be no longer than 45 minutes, as the carbohydrates will start to break down. Remove from heat and put on stir plate until agar is cooled down enough to pour safely. Lay out petri dishes and pour 4 to 5 mLs into each plate, replace lids and allow agar to solidify. Refrigerate for up to 2 weeks.

FILTER PADS

Set up filtering apparatus and attach to vacuum source. Filter sample, rinse with at least two rinses. After filtering sample, place filter on prepared mTEC plate that has been warmed to room temperature. Be careful to avoid air bubbles, pad should be in good contact with the media.

RESUSCITATION

Cover plates, and incubate for 2 hours at 35E inverted.

INCUBATION

Place in heat-sealed bag making sure that it is Watertight; place inverted into a 44E water bath for 22 hours.

SUBSTRATE PREPARATION

Prepare urea substrate using urea, phenol red and lab grade water. Adjust pH with HCL or NaOH. Urea should be bright yellow. This mixture can be stored and refrigerated for up to 7 days.

READING THE PLATES

Remove the plates from the water bath, open bag and open plates. Place an absorbent pad in each lid. Pipette approximately 2 mLs of substrate onto pad and transfer the filter onto it using forceps. Be careful not to tear the filter. After 15 to 20 minutes, using a lamp and magnification, count and record the yellow to yellow-brown colonies.

MEDIA

COLISCAN MFh media. Stored frozen safely for 6 months. Thaw as needed. Pipette approximately 1.8 mLs onto sterile pad in petri dish.

FILTER PADS

Same as mTEC method, place filter on prepared COLISCAN MF7 plate.

RESUSCITATION

None required.

INCUBATION

Place in heat-sealed bag and put in incubator at 35E for 22 hours.

SUBSTRATE PREPARATION

None required.

READING THE PLATES

Remove plates from incubator, remove lid and count colonies that are blue (*E. coli*); all other coliforms appear as pink, and non-coliform colonies appear as white or translucent.